# THE EFFECT OF IODINE AND IODOTHYRIN ON THE LARVÆ OF SALAMANDERS. III. THE RÔLE OF THE IODINE IN THE SPECIFIC ACTION OF THE THYROID HORMONE AS TESTED IN THE METAMORPHOSIS OF THE AXOLOTL LARVÆ.*

E. UHLENHUTH.

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The rôle of the iodine in the specific action of the thyroid hormone is still an undecided question. The most recent investigations on mammalia have led to the conclusion that the inorganic iodine as such is incapable of bringing about effects identical with the specific effects of the thyroid hormone.

Kendall[1] has shown that the specific reactivity of the thyroid hormone (thyroxin) as manifested by its capability of abolishing myxedema, mitigating cretinism, and raising metabolism depends chiefly on the presence, in it, of a CO-NH group. Iodine, although it may *increase* the specific reactivity of thyroxin, does not *produce* it, as is shown by the ineffectiveness of derivatives in which merely the hydrogen on the imino group has been replaced by acetyl without changing the position of the iodine atoms. Kendall, therefore, has been forced to conclude that the iodine is not responsible for the specific action of the thyroid hormone.

Quite in line with Kendall's viewpoint are Leo Loeb's recent experiments on the compensatory hypertrophy of the guinea pig's thyroid. Loeb found that the inhibitive effect in this kind of hypertrophy is specific for the thyroid hormone,[2] while inorganic iodine as such is incapable of preventing the compensatory hypertrophy of the thyroid gland. Leo Loeb, therefore, claims, like Kendall, that the effect of iodine is not identical with the effect of the thyroid hormone.

* From the Laboratories of the Rockefeller Institute for Medical Research.

1 Kendall, E. C., *Endocrinology*, 1917, i, 153–169, and 1919, iii, 156–163. Plummer, H. S., and Boothby, W. M., *Amer. J. Physiol.*, 1921, lv, 295.

2 Loeb, L., *J. Med. Res.*, 1920, xli, 481–494, and 1920, xlii, 77–89.

As Leo Loeb rightly points out, the only facts seriously in the way of his own and Kendall's viewpoint are the experiments on amphibians, especially those of Swingle,[3] which have shown that administration of inorganic iodine causes precocious metamorphosis of tadpoles. Loeb overcomes this obstacle by assuming that the amphibian metamorphosis is not so much an indicator of thyroid activity as of the action of iodine as such. Kendall[4] likewise sees himself compelled to rule the amphibian metamorphosis out of the phenomena caused by the specific action of the thyroid hormone, and assumes now that thyroxin has a twofold effect, one on myxedema and basal metabolism, which is specific to the thyroid hormone and dependent on the peculiar chemical constitution of it, and one on the amphibian metamorphosis, which is merely due to the iodine contained in the thyroid hormone and not specific for the latter one, but for the inorganic iodine as such.

In a previous article I have shown that the interpretation given by myself and others to Swingle's experiments is not correct.[5] Although it may be true that the administration of inorganic iodine enforces metamorphosis of the tadpoles, it must be kept in mind that from the *effect of the administration of a substance* one can not, with any certainty, conclude upon the *effect of the administered substance,* unless the fate which this substance undergoes in the body is known. As to the fate of the inorganic iodine in the tadpole body, there is much evidence to show that it does not produce metamorphosis in the form of inorganic iodine, but after the thyroid—or in the absence of this gland other tissues similar in their function to the thyroid gland—has elaborated from it the thyroid hormone. The results on iodine-fed tadpoles, therefore, are neither new nor are they surprising as far as the ability of the tadpole thyroid goes to produce more "active thyroid hormone," if more iodine is administered; it is long known that the mammalian thyroid possesses exactly the same ability as shown by Marine and Rogoff in quantitative experiments.[6] The only thing new in Swingle's experiments is the discovery that the thyroid apparatus

[3] Swingle, W. W., *J. Exper. Zoöl.,* 1918–19, xxvii, 397–415.

[4] Kendall, E. C., *Amer. J. Physiol.,* 1919, xlix, 136–137.

[5] Uhlenhuth, E., Endocrinology (in press).

[6] Marine, D., and Rogoff, J. M., *J. Pharm. and Exper. Ther.,* 1916, ix, 1–10.

of the tadpoles or certain parts of it do not possess the ability of storing the thyroid hormone after its elaboration. The tadpole experiments in themselves, however, do not decide anything as to the rôle of the inorganic iodine in the action of the thyroid hormone. When viewed in the light of the experiments on salamanders they support the conception that inorganic iodine as such does not play an essential rôle in the effects characteristic for the thyroid hormone, and that the amphibian metamorphosis can be effected *only* by the thyroid hormone.

I have shown[5] that inorganic iodine, when administered to such amphibians in which the control of the excretion of the thyroid hormone is similar to that in mammalians—*i.e.*, in which no hormone can escape from the thyroid in the absence of the action of a particular releasing mechanism—is completely unable to produce the amphibian metamorphosis. In the experiments to be reported below I have used so-called axolotl larvæ. The axolotls, which are the larvæ of the species *Ambystoma tigrinum*, are characterized by an inhibition of metamorphosis resulting in a considerable prolongation of the larval period. It is possible, therefore, to extend, in this species, the experiments over long periods and to administer excessively large quantities of iodine. The ineffectiveness of such large quantities of inorganic iodine as compared to the prompt effect of amounts of iodothyrin containing only minute quantities of iodine disproves, in a quantitative manner, that inorganic iodine is the active principle of the thyroid hormone in the amphibian metamorphosis.

## Experiments.

From about 100 larvæ of the salamander *Ambystoma tigrinum*, which had developed into so-called "axolotl larvæ,"[7] 9 larvæ were selected for the experiment to be reported here. They were kept in ordinary tap water and fed earthworms in the beginning, beef liver later on.

### *Beginning of Experiment (December 9, 1920).*

No. 1 measured 189.0 mm.; No. 2, 160.6 mm.; and No. 3, 194.4 mm. All three animals were completely larval (large gills, large

[7] The axolotl larvæ were collected in the Colorado Rocky Mountains in August and September, 1920.

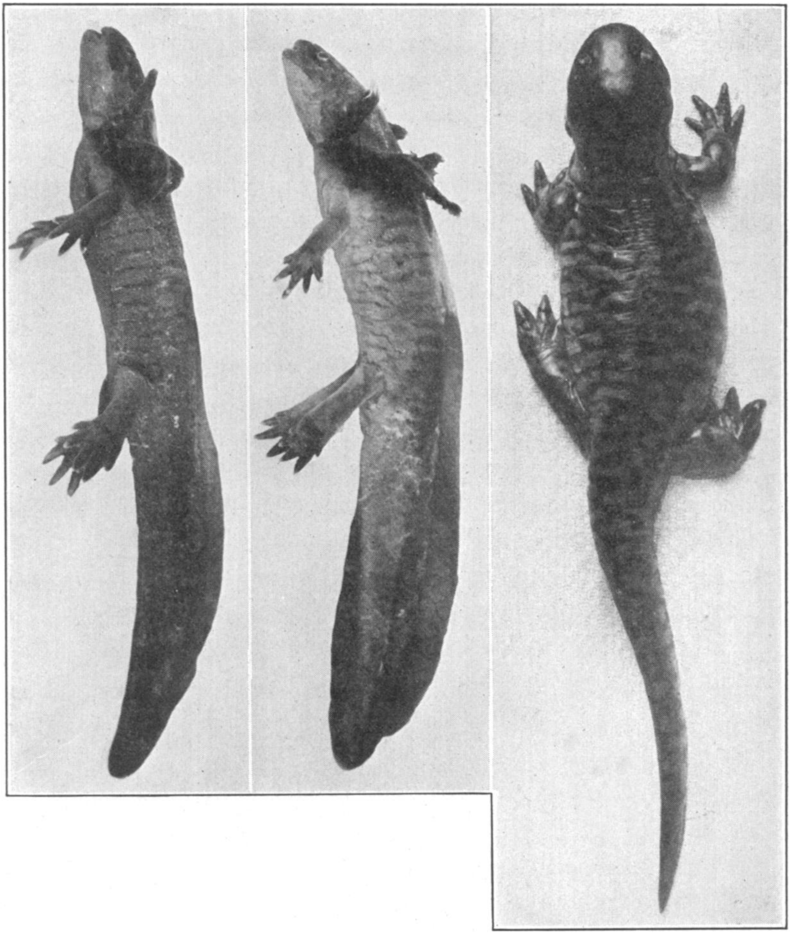

FIG. 1. Control larva 134 days after the beginning of the experiment. Note the shortened gills and the almost complete absence of the anterior portion of the ventral fin.

FIG. 2. Iodine-fed larva 134 days after the beginning of the experiment. Note the long gills and the completeness of the fin. This larva, although large amounts of inorganic iodine were administered to it, is less far advanced towards metamorphosis than the control animal.

FIG. 3. An animal to which iodothyrin was administered, photographed 134 days after the beginning of the experiment. This animal metamorphosed 13 days after the first administration of iodothyrin; it was a completely metamorphosed terrestric salamander when the photograph was made, and had been so several months before it was photographed.

fin on ventral and dorsal side of tail, eyes not bulging out) and No. 3 was neotenous (a sex mature male whose cloacal lips were swollen and warty). These 3 larvæ served as controls.

No. 4 measured 184.9 mm.; No. 5, 158.5 mm.; and No. 6, 214.0 mm. All 3 larvæ were completely larval; No. 6 was a sex mature male. This set of larvæ was used for the iodine experiment.

No. 7 measured 179.8 mm.; No. 8, 154.2 mm.; and No. 9, 184.5 mm. Again each animal was completely larval; the third one, however, presented no external sign of sex maturity (it was probably a female). This set was used for the iodothyrin experiment.

It will be noticed that each larva of each set was comparable to a corresponding larva of the two other sets, as regards size and stage of development. The larvæ selected for the iodothyrin experiment, however, were smaller in average than the larvæ of the two other sets. This selection was made in order to be sure that in case the larvæ kept in iodothyrin should metamorphose before the other larvæ their earlier metamorphosis was not due to a more advanced stage at the beginning of the experiment, but to the specific effect of the iodothyrin.

On December 9, 1920, the larvæ of the second set (Nos. 4, 5, 6) were placed into an iodine solution containing 5 drops of a 1/20 M stock solution of inorganic iodine (in 95 per cent. alcohol) per 1,000 c.c. of water, while the larvæ of the third set (Nos. 7, 8, 9) were put into water containing 0.1 gm. of Bayer's iodothyrin per 1,000 c.c. of water. The larvæ of the first set (Nos. 1, 2, 3) served as controls.

*Thirteen Days after the Beginning of the Experiment.*—The controls and the larvæ of the iodine experiment do not show any changes, in particular no signs of metamorphosis. Every one of the three larvæ kept in iodothyrin was found this morning to have shed its skin for the first time.

These three larvæ absorbed the gills very quickly, moulted several more times in rapid succession, and 25 days after beginning of the experiment had reached the stage at which the larvæ must be removed from the water and put on land, in order to prevent

drowning. Fig. 3 shows No. 9 completely metamorphosed 134 days after the beginning of the experiment.

*Forty-three Days after the Beginning of the Experiment.*—Since 5 drops of iodine per 1,000 c.c. of water had no effect on metamorphosis, the iodine concentration was gradually increased and has reached today 9 drops in No. 5 and 8 drops per 1,000 c.c. of water in Nos. 4 and 6. Since it is believed that the alcohol used as solvent of the iodine might have some influence, too, on the animals, an equal number of drops of 95 per cent. alcohol is added from now on to the water in the controls.

*Fifty-five Days After the Beginning of the Experiment.*—The larvæ of the iodine series did not show any sign of an approach towards metamorphosis. Therefore the concentration of the iodine (and of the alcohol in the control series) was further increased and reached today 13 drops per 1,000 c.c. of water, a concentration of iodine more than 4 times the concentration sufficient to cause growth of the limbs in tadpoles.

*Seventy-five Days After the Beginning of the Experiment.*—The strength of the iodine solution was kept till today at 13 drops of a 1/20 M solution of iodine per 1,000 c.c. of water; in addition one larva (No. 5) was fed iodine crystals directly per mouth (1 crystal on the 57th, 63d, 68th, 70th and 74th day after the beginning of the experiment). Yet, in spite of these large amounts of inorganic iodine being adminstered to the larvæ, none of them shows any signs of metamorphosis so far.

From now on all larvæ, controls as well as experimentals, are kept in ordinary tap water, but the larvæ, Nos. 5 and 6 of the iodine series (No. 4 is used for a different experiment) are fed crystals directly per mouth.

*One hundred and thirty-four Days After the Beginning of the Experiment.*—The total number of iodine crystals fed to each one of the iodine-fed larvæ is now 25. This number of crystals was fed to No. 5 during a period of 76 days and to No. 6 during a period of 59 days. These large amounts of inorganic iodine were incapable of accelerating metamorphosis. In both the controls and the experimentals one larva has shed its skin, the iodine larva (No. 6) being now farther advanced than the corresponding control. But No. 5, the other iodine-fed larva, is completely

larval and distinctly less far developed than the least advanced control larva No. 2.

One larva of each set was photographed today. Fig. 1 shows the least advanced control larva No. 2, Fig. 2 the least advanced iodine-fed larva No. 5, and Fig. 3 the iodothyrine-fed animal No. 9. While the latter animal is a completely metamorphosed terrestric salamander and has been so for several months, both the control and the iodine-fed animal are larvæ. The control possesses, however, slightly shorter gills, its eyes are slightly more bulging than those of the iodine-fed larva (not visible in the photograph) and the ventral portion of the fin is greatly absorbed as compared to the completely preserved fin of the iodine-fed animal. On dissection the small intestine of the control, although the control larva was larger than the iodine-fed larva, was found to be shorter (308.5 mm.) than that of the iodine--fed larva (387 mm.).

In short 134 days after the beginning of the experiment the least advanced control larva proved to be nearer metamorphosis than the least advanced iodine-fed larva, although the latter was kept, till the 75th day after the beginning of the experiment, in iodine solutions of concentrations up to 13 drops of 1/20 M solution of inorganic iodine per 1,000 c.c. of water, and in addition had received a total number of 25 iodine crystals during the last 76 days of the experiment.

## Discussion.

The experiments reported in this article fully confirm the experiments reported in a previous paper, in which I showed that inorganic iodine does not enforce metamorphosis of normal larvæ of the salamander *Ambystoma punctatum*.[5] The administration of inorganic iodine, even if this substance be administered in comparatively large doses, is incapable of bringing about the amphibian metamorphosis in those species in which the control of the excretion of the thyroid hormone is of such a kind that no hormone can escape from the thyroid before the end of the larval period.[5, 8]

[8] Uhlenhuth, E., *J. Gen. Physiol.*, 1919, i, 473–482, and *Am. Nat.*, 1921, lv, 193–221.

In the mind of the readers not quite familiar with the amphibian technique doubt may arise as to the correctness of the interpretation of the metamorphosis of one of the iodine-fed larvæ as not being due to the action of the iodine. I may mention, therefore that all of the axolotl larvæ, when kept under the conditions employed in the present control experiments, do undergo metamorphosis spontaneously. Consequently metamorphosis of the iodine-fed axlotl larvæ could be interpreted as the result of the iodine administration only, if it would have occurred at a greatly precocious date and simultaneously or nearly simultaneously in all the experimental larvæ. Not only were these conditions not fulfilled, but one of the experimental larvæ did not metamorphose at all and, at the termination of the experiment was farther away from the metamorphosis than any of the control larvæ, although observation extended over a period of more than 4 months, during which time large amounts of iodine were administered.

That the dosis of iodine was too small to enforce metamorphosis, can not be assumed; for, as will be discussed presently, the dosis of iodine administered to the larvæ of the iodine series was far in excess over the quantity of iodine contained in the dosis of iodothyrin which caused a very rapid metamorphosis.

Furthermore it is impossible to explain the ineffectiveness of the inorganic iodine by assuming that the larvæ employed in the iodine experiment had entered a stage at which the responsiveness to the stimuli causing metamorphosis has been lost. Not only could metamorphosis easily be produced by iodothyrin, but occurred spontaneously in the controls and in many other axolotl larvæ comparable to the iodine-fed larvæ in every respect (except for the iodine administration).

Hence it is evident that inorganic iodine is incapable of causing metamorphosis of the salamander larvæ.

Regarding the rôle of the iodine in the thyroid hormone these experiments also show, in a very striking manner, that the effect of the iodothyrin is not due to its iodine content. If it were due to its iodine content, quantities of inorganic iodine equal to those contained in an effective dosis of iodothyrin should cause metamorphosis. This expectation, however, is not fulfilled in the re-

sults of the present experiments. Bayer's iodothyrin, according to Bayer's "Materia Medica" and according to Baumann's own description of this substance,[9] contains 0.3 mgm. iodine per 1 gm. of substance. Consequently a dosis of 0.1 gm. of iodothyrin, which causes very rapid metamorphosis of the axolotl larvæ, contains 0.03 mgm. iodine per 1,000 c.c. of water. The weakest solution of inorganic iodine employed in the iodine experiment (5 drops of a M/20 iodine solution per 1,000 c.c. water) contained approximately 1.0 mgm. of iodine per 1,000 c.c. of water (1 drop being equal to about 0.2 mgm. of iodine), while the strongest solution contained even 2.6 mgm. iodine per 1,000 c.c. water. In addition to these quantities of inorganic iodine the larvæ of the iodine series received, directly per mouth, a total amount of 25 iodine crystals which equals approximately 40 mgm. of iodine, the average weight of one crystal being 1.6 mgm. Yet these quantities of inorganic iodine did not cause metamorphosis, although they were greatly in excess over the quantity of iodine contained in an effective dosis of iodothyrin. It is obvious that it is not the quantity of iodine contained in the iodothyrin, which produces the effectiveness of the latter substance.

Lenhart's experiments[10] showed that the effectiveness of thyroid gland in the amphibian metamorphosis of tadpoles increases with an increasing amount of iodine. These experiments are frequently quoted to demonstrate quantitatively that the active substance of the thyroid hormone in the amphibian metamorphosis is iodine. In the light of the present experiments this interpretation of Lenhart's experiments seems untenable. As pointed out in the introduction of this article, it is probable that the inorganic iodine does not produce the metamorphosis of tadpoles in its inorganic form, but after the thyroid apparatus has elaborated from it the thyroid hormone. Therefore Lenhart's experiments may be simply the expression of the fact that the tadpole thyroid, as claimed by Swingle[11] elaborates more hormone if more iodine is available. Moreover it is very probable that in Lenhart's experiments an increased amount of iodine contained

[9] Baumann, E., and Roos, E., *Zeitschr. f. physiol. Chem.*, 1895–96, 481–493.

[10] Lenhart, C. H., *J. Exper. Med.*, 1915, xxii, 739–746.

[11] Swingle, W. W., *J. Exper. Zoöl.*, 1919, xxvii, 417–425.

in the specimens of thyroid gland which were fed to the tadpoles corresponded to an increased amount of thyroid hormone (A-iodine, Kendall); in this case Lenhart's experiments would mean that metamorphosis is the more accelerated, the more thyroid hormone there is supplied to the tadpole, a result which corresponds with quantitative experiments on the action of iodothyrin in salamanders[5] and does not throw any light on the rôle of iodine in the thyroid hormone.

Swingle[12] in support of his viewpoint that inorganic iodine as such causes the amphibian metamorphosis, has emphasized the specificity of iodine, the administration of inorganic bromine being incapable of causing metamorphosis. It is not impossible, however, that this fact merely means that the organism does not possess a mechanism by means of which bromine can be employed in the manufacture of thyroid hormone; if it were possible to make synthetically, outside the organism, a substance identical with Kendall's thyroxin in every respect, but possessing a bromine atom in place of every iodine atom, this substance may be capable of producing metamorphosis.

## Summary.

1. Iodothyrin and inorganic iodine, in known quantities, were administered to so-called axolotl larvæ of the salamander *Ambystoma tigrinum*.

2. A dosis of iodothyrin containing only 0.03 mgm. iodine per 1,000 c.c. of water caused metamorphosis 13 days after its first administration.

3. A dosis of inorganic iodine 33 to 86 times larger and feeding still larger doses directly per mouth did not cause metamorphosis.

4. The amphibian metamorphosis is truly the expression of the thyroid activity and not the result of the effect of inorganic iodine.

5. Inorganic iodine as such is not the active principle of the thyroid hormone.

[12] Swingle, W. W., *J. Gen. Physiol.*, 1919, i, 593–606.